Flowing time on the peritoneal membrane

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Time, passing on, rhythms our lives. This observation also applies to physiology and pathophysiology. It is known that this seasonal adaptability is based both on genetic programmes, and on a strict neurovegetative and endocrinological control, with a flexible and sophisticated network of activities changing in relation to external stimuli and aging, starting in intrauterine life to the years of growth, adulthood and senility. Likewise, the peritoneum of peritoneal dialysis (PD) patients responds to the passing of time by undergoing anatomical and functional changes (Figure 1).

Although the functional characteristics of the peritoneum in children undergoing PD are different from those in adult patients, recent experimental studies have demonstrated that, when adjusted in relation to body surface and age, there are no significant differences in peritoneal fluids and solutes transport between adults and children. In a recent study on children under PD, time was found to have no effect on parameters for peritoneal transport, except for the restriction coefficient for macromolecules [1].

**Factors involved in peritoneal ultrafiltration failure**

A large body of data demonstrates that the risk of encountering a clinically relevant reduction in peritoneal ultrafiltration increases in relation to the time of PD, and this risk is estimated to be ~35% after 6 years of treatment [2]. Ambulatory PD (APD) or continuous ambulatory PD (CAPD) modalities do not have a significant influence on small solute transport or fluid kinetics [3]. Currently, four mechanisms are known to underlie failure in peritoneal ultrafiltration [4]. Different data demonstrate that aquaporin-mediated water transport is altered in patients under long-standing PD [5,6]. An increased peritoneal absorption of small osmotically active solutes, followed by a dramatic fall in the osmotic gradient, is a common and widely known mechanism in decreasing ultrafiltration [7]. A hypopermeable peritoneum with loss of peritoneal surface area, typically after severe peritonitis with adhesions, or in the case of sclerosing peritonitis, is probably a rare mechanism of effective ultrafiltration failure. A poor effective ultrafiltration due to high lymphatic absorption rates is also considered of particular importance in peritoneal aging [8].

**Structural and functional changes of peritoneal wall**

Various morphologic and structural alterations can occur in the peritoneal tissue, especially after long-standing PD. The most frequently described alteration in the peritoneum of patients on long-standing PD is the formation of a layer of collagenous, acellular material replacing the mesothelial surface. The peritoneum of patients with this type of alteration contains groups of cells exposed to degenerative phenomena, including intracellular oedema, destruction and degener-
eration of cytoplasmic structures and large areas of peritoneal denudation among mesothelial cells, which are still intact [3]. Also, mesothelial cell cultures with long-standing exposure to glucose solutions undergo early degenerative alterations, such as hypertrophy, intracellular oedema, cell cycle arrest, early aging and death. During CAPD, mesothelial cells undergo epithelial-to-mesenchymal transition [9]. These cells differ greatly from non-exposed mesothelial cells, in morphology and probably in function, with a strongly accelerated life cycle [10,11]. Reduplication and thickening of the basal mesothelial membrane have also been described in the peritoneum of patients on long-standing PD, and these alterations are similar to those described in patients with diabetes mellitus. The membrane, some areas of which appear fragmented, is made up of bands of collagen and rectiform elastic lamina within an amorphous matrix [12]. An increase in the fibrotic processes of the sub-mesothelial layers is a typical finding in a peritoneum with long-standing exposure to hypertonic glucose solutions. However, before dialysis is started, a significant thickening of the sub-mesothelial space is present in patients with pre-terminal uraemia. This thickening is similar to that found in peritoneal biopsies of patients who undergo haemodialysis before switching to PD. In their study on rat peritoneum, Combet et al. [13] demonstrated that such alterations are more significant 6 weeks after induction of a uraemic status compared with 3 weeks. Each structural alteration has different functional consequences [14]. Sub-mesothelial and perivascular fibrosis increases the distance between endothelium and dialysate, thus increasing permeability to small solutes. The increased peritoneal permeability observed in uraemic rats appears to depend more on vascular proliferation than on fibrosis. The increased expression of angiogenic factors VEGF and FGF2 at the third week of induction of uraemia suggests that these growth factors are involved in neovascularization and in the fibrotic alterations observed at 6 weeks [13]. Nitric oxide (NO) is essential for the biological activities of VEGF, and an increased expression of both these

Fig. 1. Light microscope: 18 years old (a–b). One micrometre-thick sections, coloured with toluidine blue. Some fields show flat mesothelial cells (a); in other fields one can observe cells becoming cubic with very tight junction between them (b) (×750). 69 years old (c). One micrometre-thick section, coloured with toluidine blue. In some areas, one can notice the absence of mesothelial cells (arrow) (×750). Transmission electron microscope: 18 years old (d). Cubic cells show very tight junction between them, a normal basal membrane and microvillous (×12 500) 69 years old (e). It is possible to observe a flat cell with a thickened basal membrane and short, rare and squat microvillous (×8200).
mediators could cause the increased vascularization found in the peritoneum of patients on long-standing PD [15]. The data suggest that uraemia itself can induce anatomical changes in the peritoneal membrane before dialysis is started, and these alterations may depend on the chronic inflammatory status typical of this condition.

The important changes in the structure of the peritoneal vessels described in PD patients, similar to those observed in patients with diabetic microangiopathy, include: reduplication of the basal capillary membrane, expansion of the extracellular matrix within the middle arteriolar sheath and type IV collagen deposits in the arteriolar walls. Several authors have found a link between loss of ultrafiltration and the severity of lesions, and suggest that vascular alterations depend on the production, and depositing, of advanced glycosylation products, AGE [16]. Williams et al. [17] found vasculopathy in 20% of peritoneal biopsies obtained from uraemic patients, not on PD. The percentage of patients with vasculopathy increases in parallel with the duration of PD; after 6 years of treatment, 87% of biopsies present clear signs of vascular damage.

**Potential therapeutic strategies**

A better understanding of these mechanisms should yield new therapeutic strategies aiming to protect the peritoneal membrane from the consequences of long-term PD [18]. Pharmacological strategies should aim to reduce RCO and AGE formation in the dialysate through solutions other than glucose and heat-sterilized ones, or through AGE formation inhibitors or L-arginine–NO pathway inhibitors, using L-arginine analogues. Another possible therapeutic strategy might consist of modulating angiogenesis using agents that inhibit endothelial cell growth, adhesion and cell migration, or that interfere with vascular growth factors VEGF and βFGF, or their receptors. The potential benefit of the above treatments should be carefully evaluated, as the inhibition of multifunctional medi-
Future perspectives

New perspectives in treatment and prevention of ultrafiltration failure are offered by gene therapy, to preserve the structure and function of the peritoneal membrane. Peritoneal mesothelial cells or peritoneal leukocytes can be modified to express anti-inflammatory cytokines, as interleukin-1 receptor antagonist (IL-1RA), the soluble receptor to TNFα and IL-10. Membrane integrity could be preserved enhancing the expression of fibrinolytic factors (tissue plasminogen activator, tPA) and anti-fibrotic molecules that counteract VEGF action and inhibit nuclear factor kappa B (NF-κB) and transforming growth factor β (TGF-β) [20] (Figure 2).

Conclusion

The peritoneum, with its simple structure but complex role, undergoes tissue damage as a consequence of different diseases. New therapeutic possibilities, respecting its morphology, will improve the maintenance of normal function over time.

Conflict of interest statement. None declared.

References